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Respuesta de la elongación foliar a la temperatura en gramíneas perennes de campo natural

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“Entre el sueño ilusorio de las distinciones rígidas y el caos paralizante de la indistinción absoluta se encuentra el mundo real de las distinciones graduales.”
Díez y Moulines

“To name and describe you must first see,
and science polished the gift of seeing.”
Robin Wall Kimmerer

“With words at your disposal, you can see more clearly.
Finding the words is another step in learning to see.”
Robin Wall Kimmerer

“One day I will find the right words, and they will be simple.”
Jack Kerouac

“[...] cualquiera que haya escrito habrá notado que al escribir siempre se despierta
algo que hasta entonces conocíamos de un modo impreciso
y que sin embargo yacía en nosotros.”
Georg Christoph Lichtenberg

“How obvious the thought seems once it has been articulated!”
Vivian Gornick

“Mi primer pan puede haber sido un fracaso, pero me lo he comido.
Por tanto, ha sido un triunfo.”
Eva Baltasar

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RESUMEN

La temperatura es un factor clave que regula el crecimiento y desarrollo de las plantas, por lo que describir sus efectos sobre las especies frecuentes puede ser relevante para entender mejor la dinámica de los ecosistemas, particularmente en sistemas biodiversos como los pastizales mixtos. Sin embargo, el conocimiento sobre la respuesta de las gramíneas a la temperatura es limitado, fuera de algunas especies cultivadas, y en las clasificaciones funcionales este aspecto se ha limitado a distinguir la vía fotosintética (C_3 o C_4). Los objetivos de este estudio fueron: modelar y comparar la respuesta de elongación foliar a la temperatura de gramíneas perennes de campo natural e identificar i) si existen respuestas distintas entre especies concurrentes con la misma vía fotosintética y ii) la temperatura de cruce entre especies C_3 y C_4. En un experimento de cámara estudiamos la elongación foliar en 14 gramíneas perennes a 6 niveles de temperatura constante. Medimos diariamente la longitud de la hoja y modelamos el efecto de la temperatura sobre la tasa de elongación foliar (TEF), el largo foliar final (LFF) y la duración de la elongación foliar (DEF), como funciones continuas. Encontramos que la respuesta de TEF a la temperatura varía entre gramíneas perennes con la misma vía fotosintética. La temperatura también afecta al LFF (relacionado con la altura de la planta) y, por tanto, a la competencia entre plantas por la luz. La temperatura de cruce media entre gramíneas C_3 y C_4 fue consistente con la predicha por el modelo de rendimiento cuántico; sin embargo, al considerar los pares individuales de especies C_3 y C_4, la variación fue muy grande (de 18 ºC a 32 ºC). La fotosíntesis no explica completamente las diferencias en la respuesta a la temperatura; hay otros factores —como el tamaño— relevantes para el crecimiento de las plantas y las interacciones con otras plantas (especialmente la competencia) y son clave para entender la dinámica de las especies de pastizales. Se encontró un patrón filogenético entre las especies Pooideae (C_3), pero en las (más recientes) Panicoideae (C_4) no fue así.

Palabras clave: Poaceae, Panicoideae, Pooideae, temperatura de cruce, vías fotosintéticas.

LEAF ELONGATION RESPONSE TO TEMPERATURE OF C_3 AND C_4 PERENNIAL GRASSES (POACEAE) FROM CAMPOS GRASSLANDS

SUMMARY

Temperature is a key factor regulating plant growth and development, thus describing its effects on species can be relevant to understand ecosystem dynamics, particularly in systems like mixed grasslands such as South American *Campos*. However, there is limited knowledge on grasses' response to temperature outside of some cultivated species and in functional classifications this aspect has been limited to the photosynthetic type. The aims of this study were to model and compare the leaf elongation response to temperature of perennial grasses from *Campos* and to identify: (i) whether there are distinct responses between co-occurring species with the same photosynthetic pathway and (ii) the crossover temperature between C_3 and C_4 species. In a chamber experiment we studied leaf elongation on 14 perennial grasses at 6 levels of constant temperature. We measured leaf length daily and modelled the effect of temperature on leaf elongation rate (LER), final leaf length (FLL) and leaf elongation duration (LED), as continuous functions. We found that the LER response to temperature varies among perennial grasses with the same photosynthetic pathway (C_3 or C_4). Temperature also affects FLL (related to plant height) and thus competition between plants for light. The average crossover temperature between C_3 and C_4 grasses was consistent with that predicted by the quantum yield model, yet when considering the individual pairs of C_3 and C_4 species, the variation was very large (from 18 ºC to 32 ºC). Photosynthesis does not completely explain the differences in the temperature response; there are other factors —such as size— relevant to plant growth and interactions with other plants (especially competition) and are key to understanding grassland species dynamics. Pooideae (C_3) species followed a clear phylogenetic pattern, but in the (more recent) Panicoideae (C_4) species this was not the case.

Keywords: Poaceae, Panicoideae, Pooideae, C_3-C_4 crossover temperature, photosynthetic pathways.

1. INTRODUCCIÓN

La temperatura regula la mayoría de los procesos vegetales, incluidos el crecimiento y el desarrollo de las plantas, por lo que es crucial para la investigación ecológica y agrícola comprender las respuestas térmicas, especialmente en el contexto del cambio climático, ya que influye en la distribución, competitividad y supervivencia de las especies. A pesar de esto, la respuesta a la temperatura se ha estudiado en detalle para pocas especies anuales cultivadas o modelo (por ejemplo, Parent et al., 2010; Parent y Tardieu, 2012; Wang et al., 2017; Yan y Hunt, 1999). Por el contrario, en el caso de las plantas que habitan en ecosistemas naturales —es decir, salvajes o nativas—, la caracterización funcional suele limitarse a simples valores resumidos, por ejemplo, la temperatura media anual de los lugares a lo largo de la distribución de las especies o simplemente la vía fotosintética (Lehmann et al., 2019).

1.1. CURVAS DE RESPUESTA COMO RASGOS DINÁMICOS O DE PROCESO

Recientemente, se ha hecho hincapié en que centrarse en rasgos estáticos limita nuestra comprensión de la función de las plantas (Anderegg, 2023; Funk et al., 2017; Jardine et al., 2020) y que se necesita un enfoque más dinámico que incluya rasgos de proceso (es decir, funciones matemáticas en lugar de valores únicos), lo que podría aportar más información (Brodribb, 2017; Kingsolver et al., 2001; Jax, 2005; Volaire et al., 2020).

Las curvas de respuesta a la temperatura pueden describir los nichos espacio-temporales en los que crecen las especies e identificar sus oportunidades para capturar y utilizar recursos por los que compiten (Fargione y Tilman, 2005; Körner, 2015). Análisis comparativos de la respuesta a la temperatura de las especies coexistentes pueden ser relevantes para comprender mejor la dinámica estacional de los ecosistemas, o incluso el efecto de futuros escenarios climáticos, especialmente en pastizales mixtos biodiversos, como los Campos sudamericanos. Estos pastizales naturales templado-subtropicales, que se extienden por Uruguay, el noreste de Argentina y el sur de Brasil, reciben precipitaciones uniformes a lo largo del año y están dominados por gramíneas perennes con fotosíntesis C_3 y C_4 (Berretta et al., 2000; Biganzoli y Zuloaga, 2015). Sin embargo, poco se sabe sobre las respuestas dinámicas a la temperatura de las especies más frecuentes y los patrones estacionales de las diferentes comunidades. Además, conocer en detalle las respuestas dinámicas de las principales especies puede ayudar a fundamentar criterios de gestión agronómica adaptativa más precisos, lo que, a su vez, puede ayudar a que estos agroecosistemas sean más sostenibles (Jaurena et al*.,* 2021).

1.1.1. Elongación foliar como variable de respuesta

El crecimiento foliar tiene varios aspectos, pero en las gramíneas la tasa de elongación foliar (TEF), la duración de la elongación foliar (DEF) y el largo foliar final (LFF) son fundamentales (Chapman y Lemaire, 1993; Lemaire y Chapman, 1996). La TEF puede utilizarse como variable de respuesta para modelizar el efecto de la temperatura sobre el crecimiento vegetativo. Es uno de los principales componentes de la formación del dosel en pastizales y, cuando el agua y los nutrientes no son limitantes, la TEF se correlaciona fuertemente con la temperatura (Ben-Haj-Salah y Tardieu, 1995; Berone et al., 2007; Duru y Ducrocq, 2000). El proceso de elongación de la hoja refleja el efecto acumulativo de la división y elongación celular, y el aumento de la longitud de la hoja a lo largo del tiempo puede modelarse como una curva logística o sigmoidea (Volenec y Nelson, 1983; Durand et al., 1999). Además, una TEF más elevada suele dar lugar a hojas más largas, por lo que no solo el potencial de crecimiento, sino también el tamaño de la planta vendrían definidos principalmente por las respuestas térmicas.

Se han utilizado muchos modelos para describir las curvas de respuesta a la temperatura, que varían en su complejidad e interpretabilidad: desde modelos lineales y segmentados simples hasta modelos no lineales (por ejemplo, funciones exponenciales y derivadas de beta, White et al., 2012; Yan y Hunt, 1999). No obstante, cuando se considera todo el rango fisiológico para una especie determinada, las curvas de respuesta a la temperatura son continuas y unimodales: alcanzan un valor máximo en un óptimo térmico y disminuyen a ambos lados hacia los límites térmicos (Duru y Ducrocq, 2000; Wooliver et al., 2022).

1.2. MÁS ALLÁ DE C_3 Y C_4

Hasta el momento no hay mayor distinción en este respecto que gramíneas invernales y estivales, que típicamente se corresponden con las vías fotosintéticas C_3 y C_4, respectivamente. El hecho de que las especies C_4 sean más comunes que las C_3 en condiciones cálidas se ha atribuido a diferencias en el rendimiento fotosintético (Ehleringer y Björkman, 1977; Ehleringer, 1978; Still et al., 2003). A altas temperaturas, la fotosíntesis C_4 es más eficiente que la C_3, debido a la mínima fotorrespiración, pero a bajas temperaturas la C_3 es más eficiente, ya que no tiene los costes energéticos extra del mecanismo de concentración de CO2. Por lo tanto, para una concentración de CO_2 atmosférico dada, existe una temperatura de cruce en la que las fotosíntesis C_3 y C_4 son igual de eficientes y que se ha utilizado para predecir la proporción global de plantas C_3 y C_4 (Collatz et al., 1998; Ehleringer et al., 1997).

Sin embargo, la fotosíntesis no es todo lo que hace al crecimiento y existen razones para creer que hay variación en las respuestas a la temperatura de las gramíneas con el mismo tipo fotosintético. Por un lado, entre las gramíneas de tipo C_3 se sabe que la subfamilia Pooideae está mejor adaptada al frío y a las heladas, lo que se refleja claramente en su distribución templada y en la evidencia de sus orígenes evolutivos (Das et al., 2021; Schubert, et al., 2019a; Vigeland et al., 2013; Zhong et al., 2018). Por otro lado, la fotosíntesis C_4 ha evolucionado de forma independiente en múltiples ocasiones dentro de las Poáceas (Christin et al., 2008; Christin et al., 2009; GPWG II, 2012; Sage et al., 2011; Vicentini et al., 2008) y se plantea la hipótesis de que la distribución de las gramíneas C_4 y su adaptación a temperaturas más altas están asociadas a sus ancestros C_3 y a las condiciones en las que evolucionaron y desarrollaron la vía C_4 (Edwards y Still, 2008; Edwards y Smith, 2010; Pau y Still, 2014). Además, aunque las gramíneas C_4 han evolucionado y se han expandido hacia climas más tropicales a un ritmo mayor que las gramíneas C_3, no se limitan a estas condiciones (Watcharamongkol et al., 2018). Por lo tanto, las diferencias en la respuesta a la temperatura entre C_3 y C_4 pueden no ser necesariamente solo un efecto intrínseco de la vía fotosintética.

1.3. HIPÓTESIS

Las hipótesis del trabajo son:

1. Gramíneas perennes coexistentes y con un mismo tipo fotosintético elongan sus hojas a diferentes temperaturas y tasas.
    1.1. Estas diferencias podrían asociarse a diferentes linajes filogenéticos.
2. Para la composición promedio de comunidades de campo natural (las especies más frecuentes), la temperatura de cruce C_3-C_4 basada en elongación foliar es consistente con el modelo de rendimiento cuántico. Sin embargo, al considerar distintos pares de especies C_3 y C_4, esta podría variar dependiendo de
    2.1. las temperaturas a las cuales responde cada una (nichos térmicos) y
    2.2. el tamaño de las plantas y la magnitud de la respuesta.

1.1. OBJETIVOS

El objetivo de este estudio fue modelar y comparar la respuesta de elongación foliar a la temperatura de gramíneas perennes, tanto nativas como exóticas naturalizadas, que coexisten en los Campos para identificar i) si existen respuestas distintas entre gramíneas con una misma vía fotosintética y ii) las temperaturas de cruce entre especies C_3 y C_4.

2. BEYOND PHOTOSYNTHESIS: LEAF ELONGATION RESPONSE TO TEMPERATURE OF CO-OCCURRING C_3 AND C_4 PERENNIAL GRASSES[1]

2.1. RESUMEN

Antecedentes y objetivos: La temperatura es un factor clave que regula el crecimiento y desarrollo de las plantas, por lo que describir sus efectos sobre las especies frecuentes puede ser relevante para entender mejor la dinámica de los ecosistemas, particularmente en sistemas biodiversos como los pastizales mixtos. Sin embargo, el conocimiento sobre la respuesta de las gramíneas a la temperatura es limitado, fuera de algunas especies cultivadas, y en las clasificaciones funcionales este aspecto se ha limitado al distinguir la vía fotosintética (C_3 o C_4). Los objetivos de este estudio fueron modelar y comparar la respuesta de elongación foliar a la temperatura de gramíneas perennes de los Campos e identificar i) si existen respuestas distintas entre especies concurrentes con la misma vía fotosintética y ii) la temperatura de cruce entre especies C_3 y C_4.

Métodos: En un experimento de cámara estudiamos la elongación foliar en 14 gramíneas perennes a 6 niveles de temperatura constante. Medimos diariamente la longitud de la hoja y modelamos el efecto de la temperatura sobre la tasa de elongación foliar (TEF), el largo foliar final (LFF) y la duración de la elongación foliar (DEF), como funciones continuas.

Resultados principales: Encontramos que la respuesta de TEF a la temperatura varía entre gramíneas perennes con la misma vía fotosintética. La temperatura también afecta al LFF (relacionado con la altura de la planta) y, por tanto, a la competencia entre plantas por la luz. La temperatura de cruce media entre gramíneas C_3 y C_4 fue consistente con la predicha por el modelo de rendimiento cuántico; sin embargo, al considerar los pares individuales de especies C_3 y C_4, la variación fue muy grande (de 18 ºC a 32 ºC).

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[1] Artículo a enviar a la revista Annals of Botany.

Conclusiones: La fotosíntesis no explica completamente las diferencias en la respuesta a la temperatura; hay otros factores —como el tamaño— relevantes para el crecimiento de las plantas y las interacciones con otras plantas (especialmente la competencia) y son clave para entender la dinámica de las especies de pastizales. Se encontró un patrón filogenético entre las especies Pooideae (C_3), pero en las (más recientes) Panicoideae (C_4) no fue así.

Palabras clave: Poaceae, Panicoideae, Pooideae, temperatura de cruce, vías fotosintéticas

2.2. SUMMARY

Background and aims: Temperature is a key factor regulating plant growth and development, thus describing its effects on species can be relevant to understand ecosystem dynamics, particularly in systems like mixed grasslands such as South American *Campos*. However, there is limited knowledge on grasses' response to temperature outside of some cultivated species and in functional classifications this aspect has been limited to the photosynthetic type. The aims of this study were to model and compare the leaf elongation response to temperature of perennial grasses from *Campos*, and to identify: (i) whether there are distinct responses between co-occurring species with the same photosynthetic pathway, and (ii) the crossover temperature between C_3 and C_4 species.

Methods: In a chamber experiment we studied leaf elongation on 14 perennial grasses at 6 levels of constant temperature. We measured leaf length daily and modelled the effect of temperature on leaf elongation rate (LER), final leaf length (FLL) and leaf elongation duration (LED), as continuous functions.

Key results: We found that the LER response to temperature varies among perennial grasses with the same photosynthetic pathway (C_3 or C_4). Temperature also affects FLL (related to plant height), and thus competition between plants for light. The average crossover temperature between C_3 and C_4 grasses was consistent with that predicted by the quantum yield model, yet when considering the individual pairs of C_3 and C_4 species the variation was very large (from 18ºC to 32ºC).

Conclusions: Photosynthesis does not completely explain the differences in the temperature response; there are other factors —such as size— relevant to plant growth and interactions with other plants (especially competition) and are key to understanding grassland species dynamics. Pooideae (C_3) species followed a clear phylogenetic pattern, but in the (more recent) Panicoideae (C_4) species this was not the case.

Keywords: Poaceae, Panicoideae, Pooideae, C_3-C_4 crossover temperature, photosynthetic pathways

2.3. INTRODUCTION

Temperature regulates most plant processes, including growth and development. In consequence, understanding thermal responses is crucial in ecological and agricultural research, particularly in the context of climate change, as it impacts the distribution, competitiveness and survival of species. Despite this, temperature responses have been studied in detail only for a few cultivated and model annual species (*e.g*. Yan and Hunt 1999; Parent *et al.* 2010; Parent and Tardieu 2012; Wang *et al.* 2017). Conversely, for plants that inhabit natural ecosystems, functional characterisation is often limited to simple summary values, for instance the mean annual temperature of sites along species' distribution, or the photosynthetic pathway (Lehmann *et al.* 2019). Recently, it has been emphasised that focusing on static traits limits furthering our understanding of plant function (Funk *et al.* 2017; Jardine *et al.* 2020; Anderegg 2023) and that a more dynamic approach including 'process' traits (*i.e.* mathematical functions rather than single values) is needed, which could yield more insights (Kingsolver *et al.* 2001; Jax 2005; Brodribb 2017; Volaire *et al.* 2020).

Temperature response curves can describe the spatio-temporal niches in which species grow and identify their opportunities to capture and use resources for which they compete (Fargione and Tilman, 2005; Körner 2015). Comparative analyses of temperature response of co-occurring species can be relevant to better understand seasonal ecosystem dynamics, or the effect of future climatic scenarios, particularly for transitional environments as is the case for highly diverse mixed grasslands such as Campos grasslands in South America. Extending over Uruguay, northeastern Argentina and southern Brazil, these temperate-subtropical natural grasslands receive rainfall evenly throughout the year and are dominated by perennial grasses both with C_3 and C_4 photosynthesis (Berretta *et al.* 2000; Biganzoli and Zuloaga 2015). However, little is known about the dynamic responses to temperature of dominant species and the seasonal patterns of different communities. Furthermore, understanding in detail the dynamic responses of the main species can help inform more accurate adaptive agronomic management criteria, which, in turn, can aid in making these agroecosystems more sustainable (Jaurena *et al.* 2021).

There are various aspects to leaf growth, but in grasses and at the organ level leaf elongation rate (LER), leaf elongation duration (LED) and the resulting final leaf length (FLL) are key (Chapman and Lemaire 1993; Lemaire and Chapman 1996). LER can be used as the response variable to model the effect of temperature on vegetative growth. It is one of the main components of canopy formation in grasslands, and, when water and nutrients are not limiting, LER correlates strongly with temperature (Ben-Haj-Salah and Tardieu 1995; Duru and Ducrocq 2000; Berone *et al.* 2007). The process of leaf elongation reflects the cumulative effect of cell division and elongation, and the increase in leaf length through time can be modelled as a logistic or sigmoid curve (Volenec and Nelson 1983; Durand *et al.* 1999). Furthermore, higher LER would typically result in longer leaves, therefore not only growth potential but also plant size would be primarily defined by thermal responses.

Many models have been used to describe temperature response curves, varying in their complexity and interpretability: from simple linear and segmented models to continuous non-linear models (*e.g*. exponential and beta derived functions; Yan and Hunt 1999; White et al. 2012). Nonetheless, when considering the whole physiological range for a given species, temperature response curves are continuous and unimodal: they reach a maximum value at a thermal optimum and decrease on both sides towards the thermal limits (Duru and Ducrocq 2000; Wooliver *et al.* 2022).

As yet there is no further distinction than cool-season and warm-season grasses, typically corresponding to C_3 and C_4 photosynthetic pathways, respectively. In grasslands, the fact that C_4 species are more common than C_3 species in warm conditions has been ascribed to differences in photosynthetic performance (Ehleringer and Björkman 1977; Ehleringer 1978; Still *et al.* 2003). At high temperatures, C_4 photosynthesis is more efficient than C_3 due to minimal photorespiration, but at low temperature C_3 is more efficient as it does not have the extra energy costs of the CO_2 concentrating mechanism. Therefore, for a given ambient CO_2 concentration, there is a 'crossover' temperature at which C_3 and C_4

photosynthesis are equally efficient, and which has been used to predict the overall ratio of C_3 and C_4 plants (Ehleringer *et al*. 1997; Collatz *et al.* 1998).

However, photosynthesis is not all there is to growth and there are reasons to believe that there is variation in the temperature responses of grasses with the same photosynthetic type. On the one hand, among C_3 grasses, the Pooideae subfamily is known to be better adapted to cold and frost, clearly reflected in its temperate distribution and evidence of its evolutionary origins (Vigeland *et al.* 2013; Zhong *et al.* 2018; Schubert, *et al.* 2019a; Das *et al.* 2021). On the other hand, C_4 photosynthesis has evolved independently multiple times within the Poaceae (Vicentini *et al.* 2008; Christin *et al.* 2008; Christin *et al.* 2009; Sage *et al.* 2011; GPWG II 2012) and it is hypothesised that the distribution of C_4 grasses and their adaptation to higher temperatures are associated with their C_3 ancestors and the conditions in which they evolved and developed the C_4 pathway (Edwards and Still 2008; Edwards and Smith 2010; Pau and Still 2014). Furthermore, although the C_4 grasses have evolved and expanded to more tropical climates at a higher rate than C_3 grasses, they are not limited to these conditions (Watcharamongkol *et al.* 2018). Thus, differences in the temperature response between C_3 and C_4 may not necessarily be just an intrinsic effect of the photosynthetic pathway.

The aim of this study was to model and compare the leaf elongation response to temperature of perennial grasses, both native and naturalised exotics, that co-occur in the South American *Campos* grasslands to identify (i) whether there are distinct responses among grasses with the same photosynthetic pathway and (ii) the crossover temperatures between C_3 and C_4 species.

2.4. MATERIALS AND METHODS

2.4.1. Experimental setup

The experiment took place at the Tacuarembó campus of the Instituto Nacional de Investigación Agropecuaria (INIA) between December 2021 and May 2022. Fourteen perennial grasses (Table 1) were grown at six levels of constant temperature from 13 to 33 ºC in two growth chambers (Sanyo Electric Co., Ltd., Osaka, Japan) arranged in a nested completely random design.

2.4.1.1. Species

Out of the 14 species (Table 1), 12 were native to *Campos* grasslands, frequent and relatively abundant and that usually co-occur, and two were naturalised exotics: *Cynodon dactylon*, the most frequent invasive species in this biome (Bresciano *et al.* 2014; García *et al.* 2023), and *Festuca arundinacea,* the most common sown forage grass species in this region (INASE 2024).

Table 1. Studied species.

Photosynthetic pathway	Species	Code name
C_3	*Bromus auleticus* Trin. ex Nees	BROAUL
	Festuca arundinacea Schreb cv. 'INIA Fortuna' (syn. *Lolium arundinaceum* (Schreb.) Darbysh.)	FESARU
	Poa lanigera Nees	POALAN
	Stipa charruana Arechav. (syn. *Nassella charruana* (Arechav.) M.E. Barkworth)	STICHA
	Stipa neesiana Trin. and Rupr. (syn. *Nassella neesiana* (Trin. and Rupr.) Barkworth)	STINEE
C_4	*Andropogon lateralis* Nees	ANDLAT
	Axonopus affinis Chase (syn. *Axonopus fissifolius* (Raddi) Kuhlm)	AXOAFF
	Coelorachis selloana (Hack.) Camus (syn. *Rottboellia selloana* Hack.)	COESEL
	Cynodon dactylon (L.) Pers.	CYNDAC

Erianthus angustifolius Nees (syn. *Saccharum angustifolium* (Nees) Trin.)	ERIANG
Paspalum dilatatum Poir.	PASDIL
Paspalum notatum Flüggé	PASNOT
Paspalum plicatulum Michx.	PASPLI
Paspalum quadrifarium Lam.	PASQUA

Plants of native species and of *C. dactylon* were collected in October 2022 at INIA's research stations "Glencoe" (-32.006931, -57.134825), "Tambores" (-31.911469, -56.227301) and "La Magnolia" (-31.70233, -55.82595). *F. arundinacea* cv. INIA Fortuna plants were collected at INIA "La Estanzuela" research station (-34.33755, -57.68529). In all cases, mature, tillered plants were taken along with a block of soil (from a single location for each species) and transported to a nursery at the INIA Tacuarembó campus.

Six groups of 1-5 tillers taken from the collected plants were transplanted to 4.5 L pots (14 cm x 16 cm x 20 cm), filled with 2 kg of a substrate with 9.8% organic matter and a pH of 6.4. After ensuring a successful transplant, the tillers were thinned and the leaf blades were cut to 5 cm. In the case of *Paspalum quadrifarium*, the smaller tillers failed to transplant and slightly bigger tillers with longer pseudostems were used. All reproductive tillers were discarded. The pots were transferred to the growth chambers for 5-12 days and fertilised with 250 ml of a complete mix containing 63 mg of N, 22 mg of P and 62 mg of K, as well as micronutrients, to complement the substrate. The day before the measurements began, three tillers with a new growing leaf were selected per pot and the unselected tillers were cut.

2.4.1.2. Growth conditions

Chamber air temperature and humidity were recorded hourly with a data-logger. The mean treatment temperatures were 13, 17, 20, 23, 28 and 33 ºC, with a standard deviation (s.d.) of 1 ºC. Full spectrum LED grow lights (HL05-200, Atop Lighting, Shenzhen, China) provided 550-700 μmol m^{-2} s^{-1} of photosynthetically active radiation at canopy level (35-55 cm from the lights) over a 12 h photoperiod.

The pots were weighed before measuring leaf length and were watered whenever soil available water content dropped below 70%. After completing the measurements, the pots were repositioned systematically each day.

2.4.1.3. Measurements

Leaf blade length (mm) was measured daily (but every 48 hours in the 13 °C treatment) using a ruler, for 11 to 25 days depending on the temperature treatment, as the distance from the tip of the leaf to the ligule of the last fully expanded leaf. Due to internode elongation, the reference on *C. dactylon* varied between successive leaves. Measurements from tillers that started elongating or flowering during the experiment were discarded.

2.4.2. Data analysis

All data was handled and analysed with R version 4.1.2 (R Core Team, 2021). The packages used were: *tidyverse* (Wickham and RStudio 2023) for data handling, iteration and plotting; *car* (Fox *et al.* 2023) for delta-method; *rsample* (Frick *et al.* 2022) for resampling, *nlme* (Pinheiro and Bates 2000) and *nlraa* (Miguez *et al.* 2022) for non-linear modelling; *lmerTest* (Kuznetsova *et al.* 2020) for linear mixed modelling; *emmeans* (Lenth *et al.* 2023) and *multcomp* (Hothorn *et al.* 2023) for post-hoc testing.

2.4.2.1. Leaf elongation model

For each measured leaf, a logistic curve was fit to the sequence of leaf blade lengths in time using a self-starting function (*SSlogis*, Eq. 1; Pinheiro and Bates, 2000):

$$L_{(x)} = \frac{FLL}{1 + e^{\frac{x_m - x}{s}}} \qquad \text{Eq. 1}$$

where $L_{(x)}$ is the leaf blade length on day, *FLL* is the asymptotic term that corresponds to the final leaf length (mm), x_m is the time of the inflection point (day), and *s* represents the time (in days) between leaf length is between 50% (inflection point) and 75% of the FLL and was used as a proxy for LED. LER (mm/day) was estimated for the central phase of rapid growth (the maximum rate and assumed constant along) from the coefficients of the fitted curve for each leaf as:

$$LER = \frac{0.25 * FLL}{s}$$

Eq. 2

The standard errors (s.e.) and confidence intervals (95%) of LER were calculated via the deltaMethod function from the *car* package (Fox *et al.* 2023). Estimates with s.e. > 5 (and s.e. > 2.5 at 13 ºC) were discarded. For the temperature response curve modelling, the s.e. of the LER estimates were included through simulated data points.

2.4.2.2. Temperature response models of LER, FLL and LED

Various models were evaluated to fit the LER response to temperature: linear-plateau, exponential, exponential-plateau, bilinear, logistic, 5-parameter-beta, using self starting functions from the *stats* (R Core Team, 2021) and *nlraa* (Miguez 2022) packages, and also the (not self-starting) 4-parameter (Yin *et al.* 1995) and 3-parameter beta (Yan and Hunt 1999). They were fit through non-linear least-squares (*nls* function) and bootstrapped (n = 100) to evaluate the rate of convergence (number of successful iterations), visual assessment of the fit and stability among iterations (normality of parameter estimates and variability in the extrapolated values).

The response was modelled with a logistic function (Eq. 1) for all species. In this case, (1) the asymptote represents the maximum LER obtained under the experimental conditions, (2) the inflection point (x_m) represents the temperature at which each species reacts the most to changes in temperature and (3) the parameter *s* defines the temperature range around the inflection point over which LER responds linearly to temperature: the linear response range ($x_m \pm s$).

The curves were fit as a hierarchical model using the *nlme* function and grouping by species, and the variance was modelled with the *varPower* function (both from the *nlme* package, Pinheiro and Bates 2022). Residuals were inspected visually through diagnostic plots, and model stability and parameter uncertainty were assessed through bootstraps of the fits (n = 1000).

Based on the bootstrapped models, temperature response groups were determined through hierarchical clustering (*hclust* function; R Core Team, 2021) using centroid linkage (UPGMC) on a matrix of the Euclidean distances of: (1) the estimates of the bounds of the linear response range and (2) the predicted LER between 10 ºC and 35 ºC (with 0.25 ºC increment steps). This allows for two levels of analysis: (1) an intrinsic or relative response that considers just the temperature axis and (2) an apparent or absolute response that integrates size effects. A decision tree (*rpart*; Therneau and Atkinson, 2023) was used to detect the threshold temperatures between clusters based on the linear response range.

The effect of temperature on FLL and LED was evaluated through linear mixed effects models (*lmer* from the *lmerTest* package; Kuznetsova *et al.* 2020) with the logarithm of FLL and *s* (a proxy for LED) as dependent variables and temperature and the logarithm of temperature respectively in interaction with the species as fixed effects. To account for the nested nature of the experimental design, random effects included tiller nested within the pot nested within the temperature level. The slopes were compared through a post-hoc test (Lenth *et al.* 2023; Hothorn *et al.* 2023). Statistical significance was tested with $\alpha = 0.05$, and model assumptions (normality and homogeneity of variance) were checked through diagnostic plots for all models.

2.4.2.3. C_3-C_4 *crossover temperature*

Using the fitted models, LER were simulated for each species over a temperature gradient ranging from 10 ºC to 35 ºC (with 0.1 ºC increment steps) and crossover temperatures determined as the lowest temperature at which leaves of C_4 species had the same or higher LER than C_3 species, for all pairs of C_3 and C_4 species (n = 45). The mean crossover temperature was estimated using bootstraps (n = 10000) for all the species and for native species only.

2.5. RESULTS

2.5.1. Co-occurring grasses with the same photosynthetic pathway respond to different temperatures

2.5.1.1. Leaf elongation rate

As expected, C_3 and C_4 species responded differently to temperature (Figure 1), both in terms of the breadth of the response and the maximal LER achieved. At temperatures below 20 ºC, leaves of the C_3 species elongated at a higher rate than those of the C_4 species. Indeed, at 13 ºC the leaves of C_4 barely elongated at all (< 2 mm/day). Conversely, the higher temperatures evaluated (28 and 33 ºC) reduced the LER of the C_3 species, while C_4 species reached the overall highest LER. Yet our results clearly show that there is more to the temperature response of LER than photosynthetic pathways.

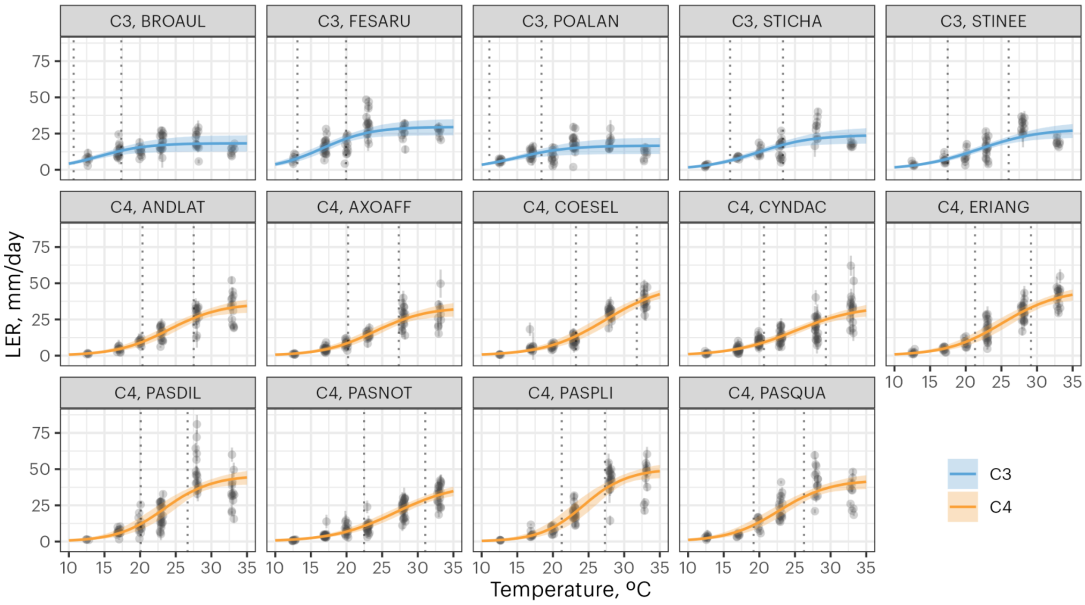

Figure 1. Fit of the logistic model to the LER estimates (points with error bars for

95% confidence intervals). Continuous line: model fit per species, bands: 95% confidence interval. Dotted vertical lines mark the linear response range (means).

Based on the bounds of linear response range (Figure 2), clear groups were found by dividing the bootstrapped estimates 1 to 5 times (2 to 6 clusters, Figure S2); further division yielded no new insights. The greatest differences were found between the C_3 and the C_4 species, as well as among the C_3. The C_4 were more monolithic than the C_3 and clusters were not as clearly divided. Four clusters were chosen as the best number for interpretation, as the C_3 and C_4 are neatly differentiated and there are two groups within each photosynthetic type with responses to higher or lower temperature. Further distinctions were too subtle to be relevant.

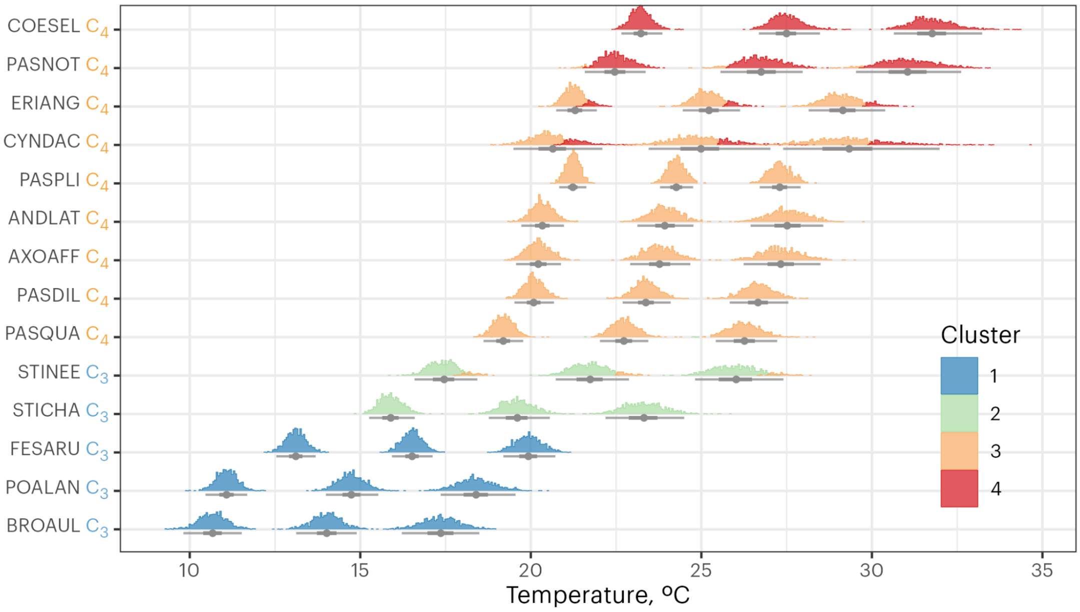

Figure 2. Density plots of the bootstrapped (n = 1000) upper bound, inflection point and lower bound of the linear response range for each species. Points represent the bootstrapped mean, the horizontal bars the 95% interval and the thicker segment the quartiles.

C_3 species presented lower bounds below 18 ºC, while C_4 species were all above. Among the C_3 species, *Bromus auleticus*, *Festuca arundinacea* and *Poa lanigera* (Cluster 1) responded to lower temperatures than *Stipa charruana* and *Stipa neesiana* (Cluster 2); the threshold temperatures were 14.5 ºC for the lower bound and 18 ºC for the inflection point. Differences among the C_4 are less marked than among the C_3, still two groups can be discerned based on their upper bounds: *Coelorachis selloana* and *Paspalum notatum* (Cluster 4) responded to higher temperatures than *Andropogon lateralis*, *Axonopus affinis*, *Cynodon dactylon*, *Erianthus angustifolius*, *Paspalum dilatatum*, *Paspalum quadrifarium*, *Paspalum plicatulum* (Cluster 3); the threshold temperature was 30 ºC for the upper bound.

When grouping the predicted LER responses (Figures 4A and S3), more clusters/distinctions were needed to account for the increased variation that is included with the size effects. Six clusters seemed like the best number: with three clusters for each photosynthetic type, and the distinction between C_3 and C_4 species was maintained as well. The C_3 remained basically the same, with the exception that *F. arundinacea* is singled out, given that it achieved higher LER values than *B. auleticus* and *P. lanigera* along the studied temperatures. Among the C_4 a different grouping emerged: (Cluster 4) *P. plicatulum*, *P. quadrifarium*, *P. dilatatum*; (Cluster 5) *A. lateralis*, *A. affinis, C. dactylon, E. angustifolius; and* (Cluster 6) *C. selloana, P. notatum.*

2.5.1.2. Final Leaf Length and Leaf Elongation Duration

The effect of temperature on FLL and LED as part of the LER response varies along the temperature gradient (Figure 3, S4 and S5). Below the lower bound of the linear response range, the leaves remain a similar (smaller) size and LED shortens rapidly with temperature. Along the linear response range, leaves become increasingly longer with temperature, while LED continues to decrease at a slower rate. The combination of both effects is part of the high responsiveness to temperature along the linear response range. Past the upper bound of the linear response range, LED remains practically fixed and the LER response results in increasingly longer leaves up to the optimum temperature.

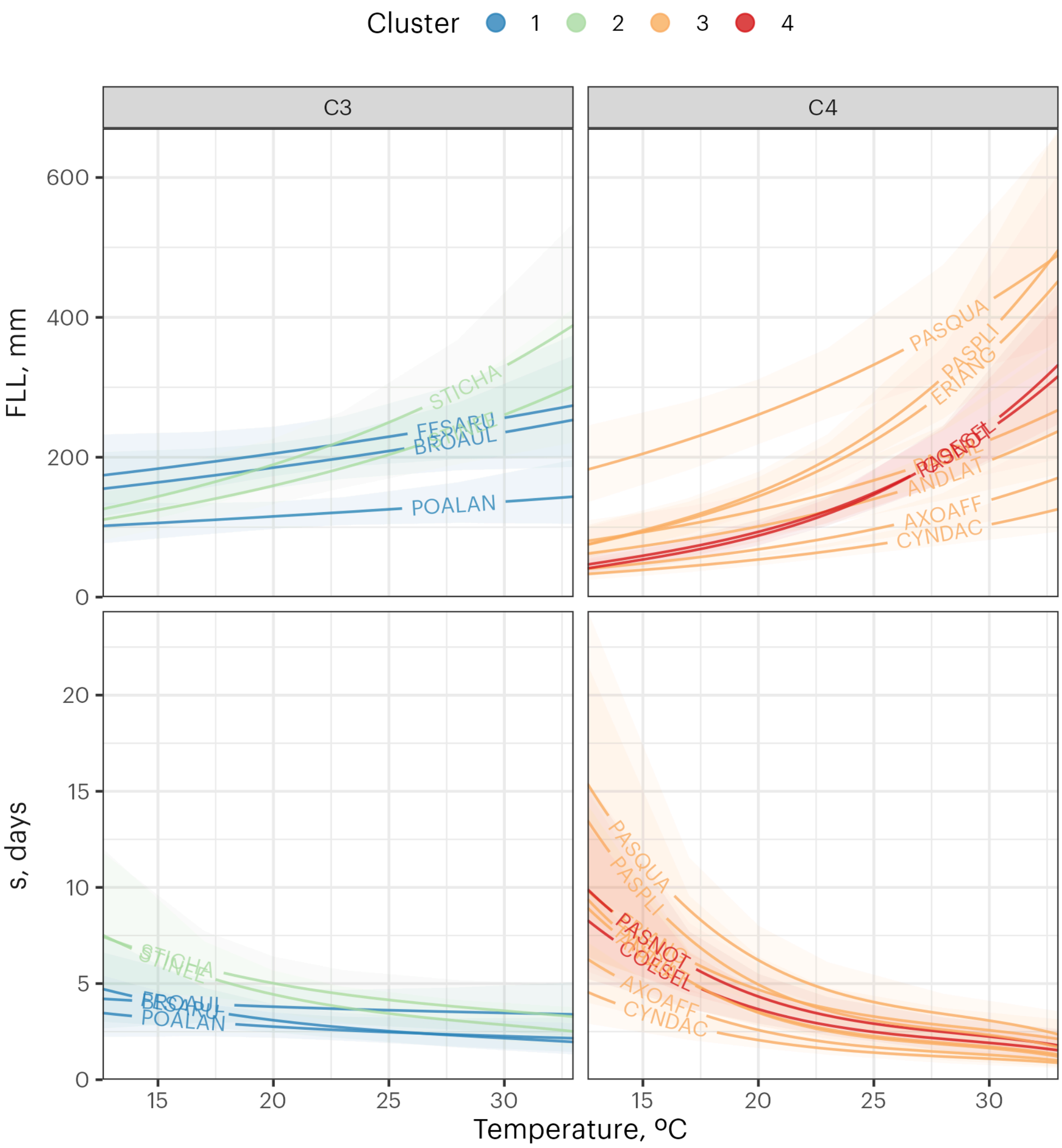

Figure 3. Prediction of the linear models for final leaf length (FLL) and the s parameter (as a proxy for leaf elongation duration) along the temperature range. The shaded area corresponds with the 95% confidence band. The colours represent the temperature response groups (clusters) for each photosynthetic pathway based on the linear response range (Figure 2).

FLL increased exponentially with temperature (Figure 3) along the studied range. The slope was statistically different from 0 for all species except *B. auleticus*, *F. arundinacea* and *P. lanigera*, and the highest responses were for C_4 species (Figure S4B). Leaf elongation duration (LED) decreased very rapidly with temperature, but the slope was indifferent from 0 for all C_3 species (Figure S5B). All fixed effects were significant (p.value < 0.05).

Just like for LER, among the C_3 both Stipa species show a delayed and steeper response to increasing temperature than the rest (*B. auleticus*, *F. arundinacea*, *P. lanigera*), for which —because of the temperature range and the limitations of the linear model— it is almost flat (Figure 3, S4 and S5). There is more variation in the C_4, and the difference in the size of these plants becomes evident and the consistencies are less clear.

2.5.2. Crossover temperature varies widely between pairs of C_3 and C_4 grasses

The mean C_3-C_4 crossover temperature was 23 ºC (21.2 ºC-24.8 ºC) considering just the natives and 24.3 ºC (22.4 ºC-26.1 ºC) including the exotics (Figure 4C). Notably, the estimates for the pairs of C_3 and C_4 species (Figure 4) ranged over 10 ºC (15 ºC including the exotics): from 18 ºC (*P. quadrifarium* and *S. neesiana*) to 28 ºC (*P. notatum* and *S. neesiana*) and 32 ºC (*C. dactylon* and *F. arundinacea*). The C_4 that crossed over at the lowest temperatures were consistently *P. quadrifarium*, *P. dilatatum*, *P. plicatulum* and *E. angustifolius*, followed by *A. lateralis* and *C. selloana*, and lastly *A. affinis*, *P. notatum* and *C. dactylon* crossed over the C_3 at the highest temperatures. The C_3 that were crossed over at the lowest temperatures were *S. charruana* and *S. neesiana*. In contrast, *F. arundinacea* was ‘first’ crossed over at temperatures above 25 ºC.

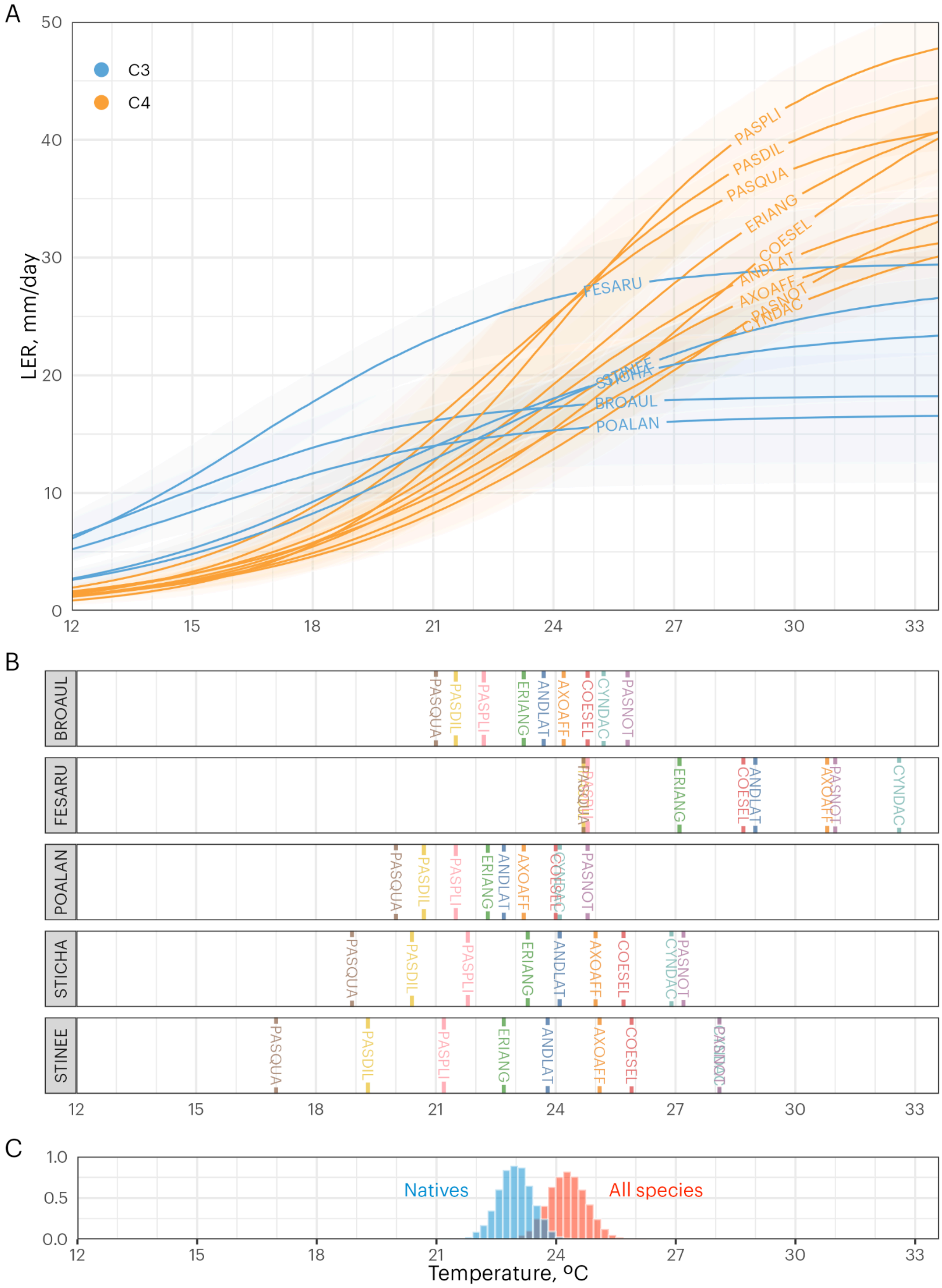

Figure 4. Overlay of the fitted curves for each species (A) and the crossover temperature of the 45 pairs of C_3 and C_4 species (B). Dotted lines indicate the limits

of the studied temperature range (13-33 ºC). Density plots of the bootstrapped mean crossover temperature for the natives and including the exotics (C).

2.6. DISCUSSION

Temperature affects virtually every plant process, yet wild perennial grasses are usually just lumped together by their photosynthetic pathway; nevertheless, there is reason to believe that it is not the sole defining factor in the temperature response. We fit temperature response curves on 14 perennial grasses from South American *Campos* grasslands based on leaf elongation and found that (1) there are different responses even among species with the same photosynthetic pathway and (2) that the C_3-C_4 crossover temperature is highly variable when considering leaf elongation rate. Furthermore, modelling the responses as continuous functions (dynamic or process traits) allows not just for a thorough description of each species' behaviour, but also for an accurate comparison of the species and show how the differences also vary along the temperature gradient.

2.6.1. Relative and absolute responses

From our results we can distinguish two levels of response: (1) one is relative and refers to the temperatures at which a species grows (position) and how the response is modulated along this range (shape) (Figure 2, Supplementary Figure 6) and (2) the other is absolute and represents the actual magnitude of the response, influenced by the size of the plants and other interacting factors (*i.e.* light quality) (Figures 1, 3, 4A). Each becomes relevant for different purposes: the relative response for physiological and phylogenetic analyses (compare the species in terms of the meristematic activity), while the absolute response for more ecological analyses, such as to evaluate plant competition and growth-based crossover temperature.

2.6.1.1. C_3-C_4 crossover temperature

The mean crossover temperature for leaf elongation (~23-25 ºC) is consistent with that of the quantum yield model for current atmospheric CO_2 concentrations of c. 400-415 ppm (Ehleringer *et al.* 1997); thus the different photosynthetic efficiencies of the C_3 and C_4 pathways is an important effect on average (Atkinson *et al.* 2016). However, in considering the various pairs of C_3 and C_4 species other factors that affect leaf growth become relevant as well, particularly plant and leaf size.In fact, it is well known that tiller size has a strong effect on LER and FLL (Casey *et al.* 1999; Arredondo and Schnyder 2003; Berone *et al.* 2007; Kavanová *et al.* 2006). But most importantly, the C_3-C_4 crossover temperature depends on the species composition of a grassland community, meaning that the C_3-C_4 ratio has a particular seasonality for each one.

2.6.1.2. Exotics

If we were to interpret the LER responses found in this study as an approximation of a species' competitive ability (perhaps as a proxy for total above ground biomass growth rates), then we could expect *F. arundinacea* to dominate these grasslands (due to having almost consistently the highest LER), yet it does not, and *C. dactylon*, though not anticipated to thrive (because of the consistently low LER), emerges as one of the most significant invaders (Bresciano *et al*. 2014). Thus these findings, though they can help enrich our interpretation of ecological processes and differentiate effects, they do not replace other plant and environmental factors that affect growth, propagation, and plant competition in the field, and their interactions, by any means.

One of these factors is stress. This study focuses on the changes in achieved LER along non-stressful temperatures, yet in the field it is common for plants to be exposed to temperatures outside of their physiological range at some point in the year. In fact, the greatest risk to the persistence of grazed *F. arundinacea* pastures in this region is the survival of spring tillers over the summer, with the most likely culprit being heat stress (rather than drought stress as has sometimes been proposed; Jáuregui *et al.* 2017; Jáuregui *et al.* 2024).

Another factor to consider is that the role of LER in a plant's ecological strategy may be species dependent rather than equal for all grasses, while also with the environmental conditions. Although initially counterintuitive with our findings (given our previous assumption), *C. dactylon* has been shown to be a great competitor in comparison with native perennial grasses, favouring to increase aerial biomass when surrounded by neighbouring plants (García *et al.* 2023). This species exhibits a rather short phyllochron (Lemaire and Agnusdei, 2000), suggesting a strategy that prioritises producing more —yet shorter— leaves, alongside internode and stolon elongation (Cruz & Boval, 2000). Not to mention that *C. dactylon* invades disturbed systems: old agricultural fields, grasslands that have become degraded by overgrazing and grasslands overseeded with exotic legumes (*e.g. Lotus sp.*) which compete with the native warm-season perennial grasses, especially in spring (Jaurena *et al.* 2016); but it does not outcompete healthy grasslands with established, thriving native warm-season perennials. On the other hand, the structure of *F. arundinacea* plants does not favour a colonisation strategy, even though (probably as a result of breeding) it produces long leaves at a high LER as a means to grow in aerial biomass.

2.6.2. Phylogeny and the evolutionary hypothesis

One of the main hypotheses for the differences in temperature responses among plants are based on evolutionary origins and an inherited adaptation to past conditions (Edwards and Still 2008; Chirstin *et al.* 2009; Taylor *et al.* 2010; Parent and Tardieu 2012). And, in fact, all the C_3 species in the experiment belong to the Pooideae subfamily (the most numerous and also known to grow at lower temperatures than other C_3 grasses within and outside the BEP clade; Sage *et al.* 2011; Vigeland *et al.* 2013; Zhong *et al.* 2018; Schubert *et al.* 2019a; Das *et al.* 2021), while the C_4 species belong to the Panicoideae subfamily (the two richest subfamilies in these grasslands, Biganzoli and Zuloaga 2015), except for the introduced *C. dactylon* that belongs to Chloridoideae (Soreng *et al.* 2017; Gallaher *et al.* 2022). Therefore, the overall differences between the C_3 and C_4 species in this study (that C_3 respond to lower temperatures and achieve lower maximum LER values) might be attributed in part to a phylogenetic effect (Edwards and Still 2008; Edwards and Smith 2010).

Furthermore, among the C_3, the species that respond to lower temperatures (*B. auleticus*, *F. arundinacea*, and *P. lanigera*) belong to the 'Core Pooideae' clade, while the ones that respond to higher temperatures (*S. charruana* and *S. neesiana*) belong to the Stipeae tribe, which diverged c. 60 Mya (Soreng *et al.* 2017; Schubert*et al.* 2019a; Zhang *et al.* 2022). The oldest records of Pooideae in South American grasslands are from ~39 Mya (Strömberg *et al.* 2013), thus Stipeae and Core Pooideae were already on independent evolutionary paths for quite some time on their arrival to South America. The same pattern was also observed for tiller LER in the flooding *Pampa* between *S. neesiana* and other coexisting Core Pooideae species (Lemaire and Agnusdei, 2000). And, in fact, 'Core Pooideae' grasses have been shown to have a higher load and expression of genes related to cold acclimation and frost tolerance than the Stipeae (Schubert *et al.* 2019b).

Conversely, among the more recent C_4 species (the first paleo records in South America of C_4 panicoid grasses are from ~18 Mya, and it was not until around 9 Mya that C_4 grasses would become dominant in grassy biomes; Edwards *et al.* 2010; Strömberg *et al.* 2013), there were no clear phylogenetic patterns. Within the Andropogoneae tribe, *A. lateralis* responds to lower temperatures than *C. selloana* and *E. angustifolius*. Even more strikingly, *P. notatum* responds to higher temperatures than the rest of the Paspalum species in the experiment. Thus, the phylogenetic relationships do not always explain the observed differences in the growing windows for these species, contrasting with the evolutionary/phylogenetic hypothesis (Edwards and Still 2008; Edwards and Smith 2010).The pattern observed among the C_3 Pooideae (and the lack of such pattern in C_4 Panicoideae) is in line with the idea that the evolution of divergent temperature responses take a lot of time considering its complexity, requiring the coordination of many processes (Parent and Tardieu 2012; Parent *et al.* 2016). Then, how can it be that four —or at least two (*C. selloana* and *P. notatum*)— of these species present contrasting responses to the rest of the sympatric C_4 grasses? How could these two (relatively unrelated) grasses evolve in this way in such an evolutionary short period while their other closer 'relatives' did not?

This highlights the novelty of studying species that are native (and not subjected to breeding processes, Parent *et al.* 2016) and that coexist in one same environment (*i.e.* sympatric, so not associated to environmental gradients as is usually done), also frequent —even dominant in some cases—, and from a system that is underrepresented in the international literature (*Campos* grasslands).

2.6.3. Future

The differences that we have observed will express themselves more strongly or weakly depending on the conditions of each year or season, thus, future studies should test interactions with other relevant environmental variables, like water and nitrogen availability, especially at higher temperatures. In addition, extending the characterisation of plant's temperature response to a larger group of species could help understand the required evolutionary time for the differentiation of leaf (cell) growth-responses compared to the appearance of the C_4 photosynthetic syndrome. From an ecological perspective, it would be interesting to study if there is a pattern of similarity or complementarity in the temperature responses of the perennial grasses in a given community.

2.7. CONCLUSIONS

By modelling the leaf elongation response to temperature we found that, indeed, C_3 species responded to lower temperatures than C_4 species; nonetheless, these are by no means homogeneous groups in terms of temperature response. Such variations are not adequately addressed within existing functional classifications and cannot solely be attributed to phylogenetic relationships.

The most contrasting differences were among C_3 (Pooideae) species, in which the Core Pooideae species responded to lower temperatures than Stipeae species (early divergent clades). However, among the C_4 (Panicoideae) the contrasts were not as sharp and, interestingly, the two species that clearly respond to higher temperatures are from different tribes (Andropogoneae and Paspaleae). Furthermore, within the genus Paspalum, one species notably deviates from the response pattern exhibited by others in the same genus.

The temperature responses appear very different depending on how they are evaluated. Some confounding effects, particularly those related to size and the magnitude of the response, can change the grouping of the species. Recognising this becomes very relevant, particularly when considering plant-plant interactions, and is very clear in the wide range of crossover temperature estimates. Interestingly, the LER temperature responses of the two exotics appear quite counterintuitive considering their relative presence in these grasslands, reflecting how complex these systems are, and that, although temperature response is extremely important, other mechanisms are at play in the success and distribution of plant species.

Overall, these results highlight the value of dynamic or process traits in describing species function, particularly in comparative analyses across different environmental conditions.

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2.9. SUPPLEMENT

Figure S1. Relative temperature responses. Colours represent the response groups/clusters based on the linear response range.

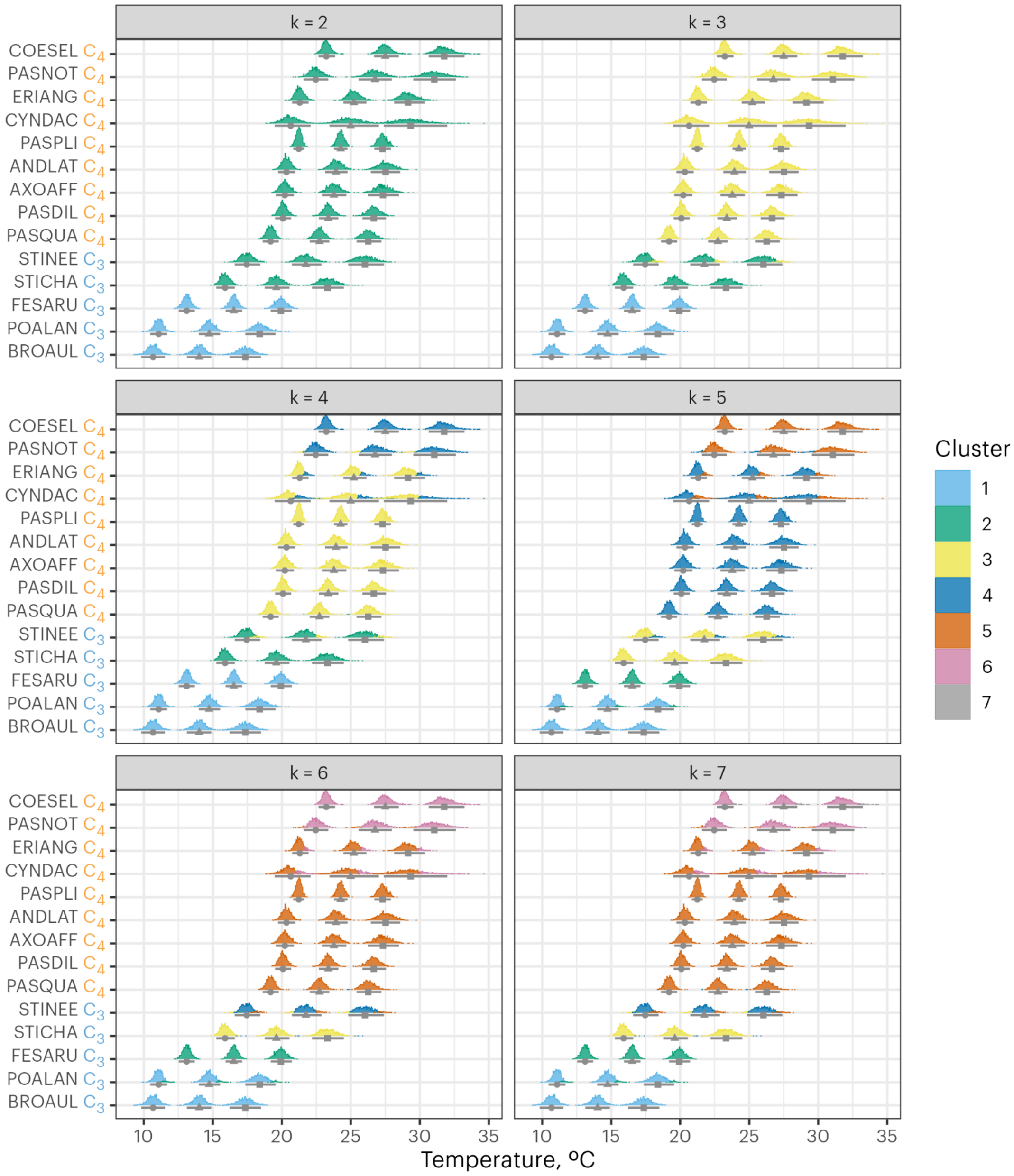

Figure S2. Hierarchical clustering of the linear response range. Each colour is a different cluster and k represents the number of clusters.

Cluster sequence:

1. First division (two clusters) separates the Core Pooideae from the rest.

2. Second division (three clusters) separates Stipeae (C3) from the C4.
3. Third division (four clusters) separates some C4 with higher temperature response (P.notatum and C. selloana).
4. A fourth division (five clusters) separates Festuca from the native Core Pooideae.
5. A fifth division (six clusters) divides the Stipa species into individual clusters.
6. A sixth division (seven clusters) does not affect the clustering at all and is thus irrelevant.

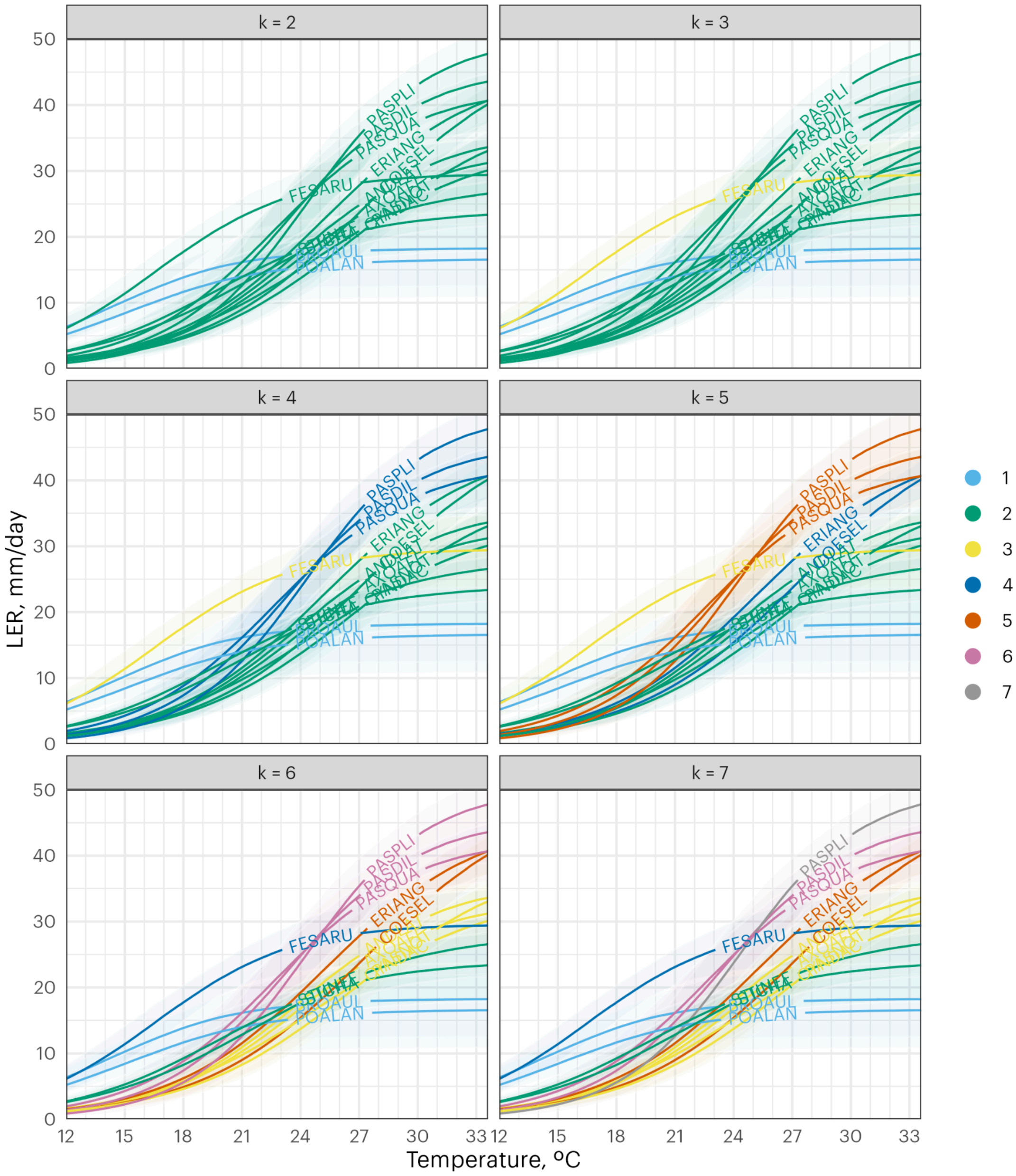

Figure S3. Hierarchical clustering of the LER response curves. Each colour is a different cluster and k represents the number of clusters.

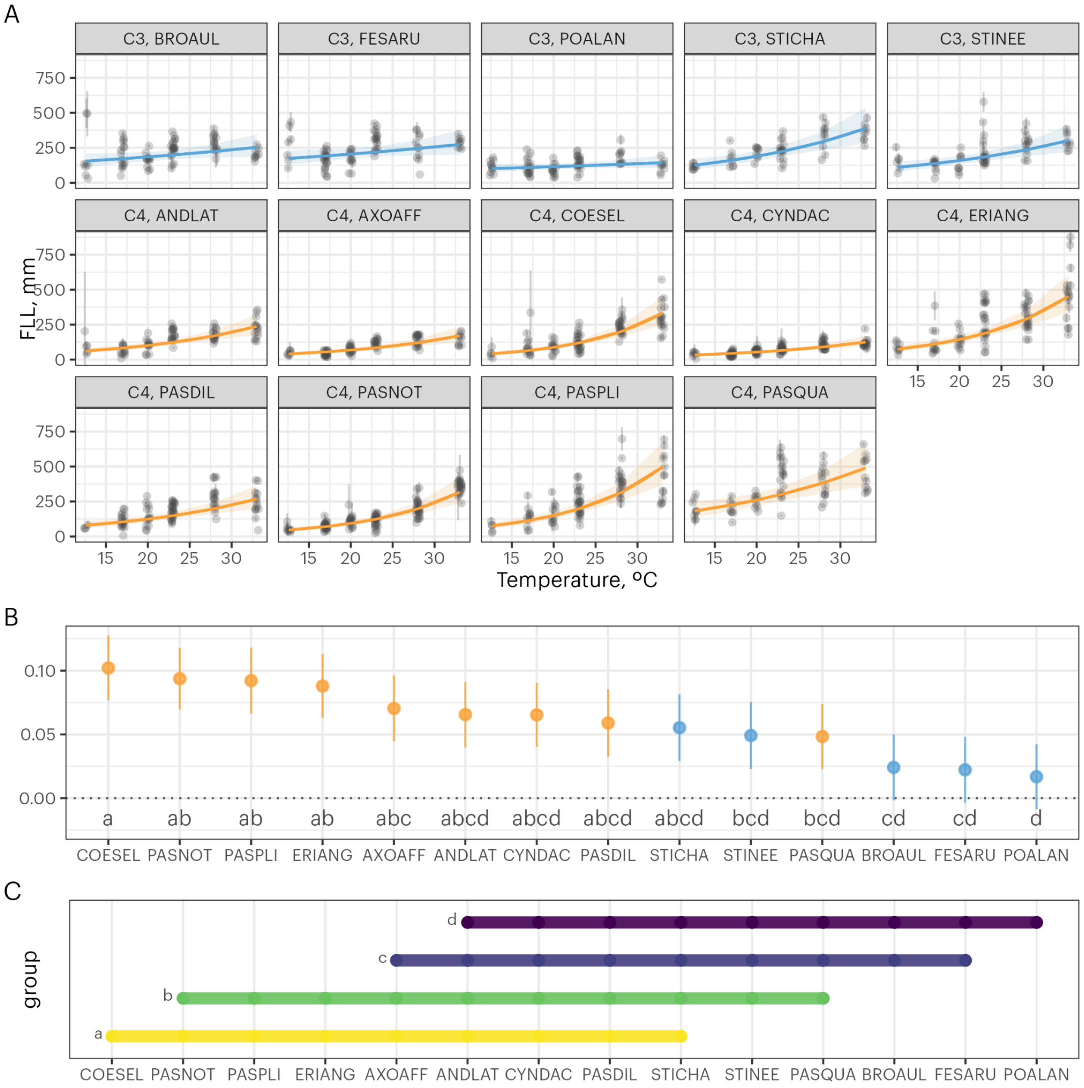

Figure S4. Linear model of the final leaf length (FLL) and the logarithm of temperature for the studied species. (A) Fit for each species. Points represent the s estimates and the shaded area corresponds with the 95% confidence band. (B) slope estimates between the logarithm of FLL and temperature for the studied species. Points represent the estimates and the error bars 95% confidence intervals. Different letters indicate significant differences between slopes (p.value < 0.05). (C) Visual representation of the letter groups.

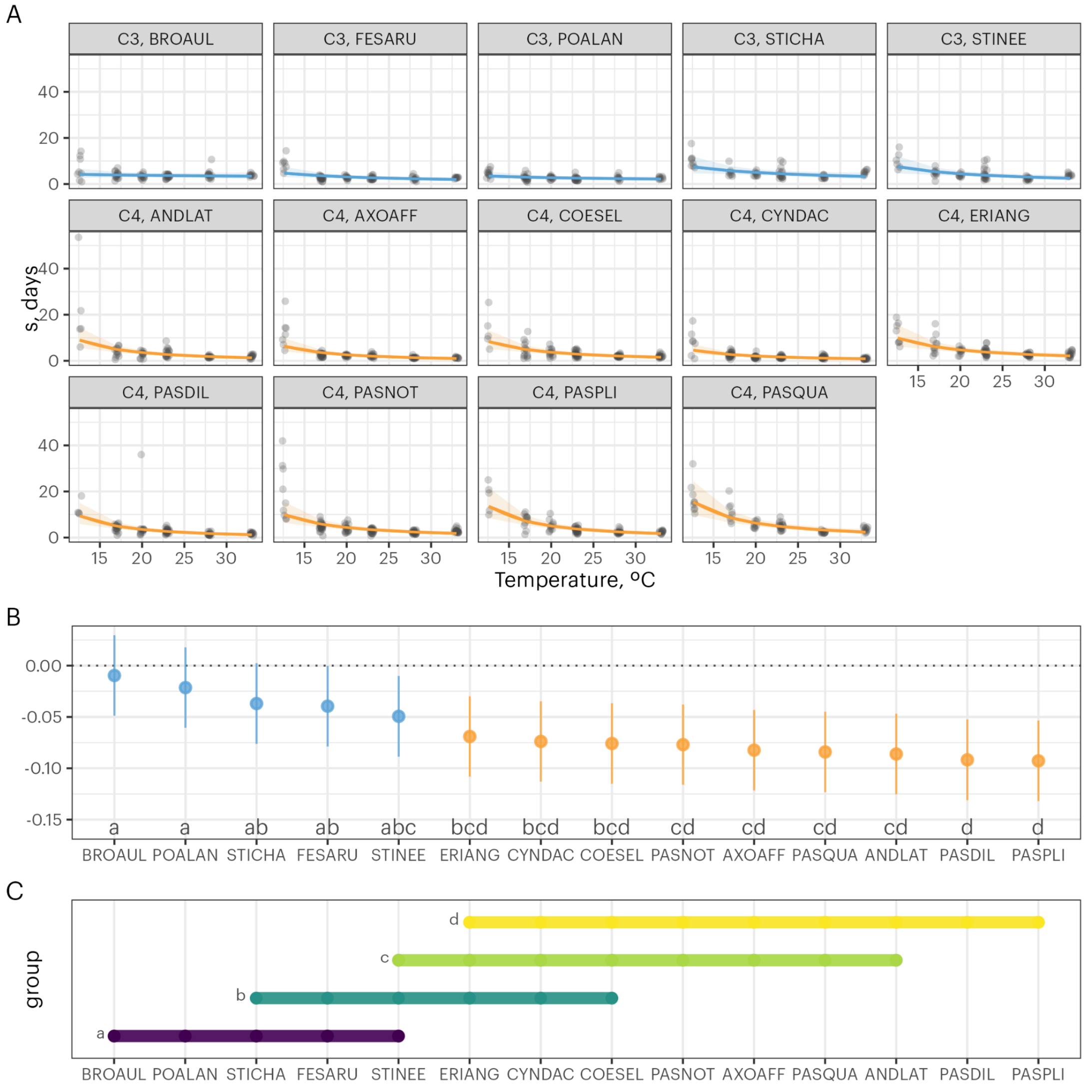

Figure S5. Linear model of the logarithm of s (as a proxy for leaf elongation duration) and the logarithm of temperature for the studied species. (A) Fit for each species. Points represent the s estimates and the shaded area corresponds with the 95% confidence band. (B) slope estimates between the logarithm of s and the logarithm of temperature for the studied species. Points represent the estimates and the error bars 95% confidence intervals. Different letters indicate significant differences between slopes (p.value < 0.05). (C) Visual representation of the letter groups.

2.9.1. Beta model for the C_3

For the C_3 species a 3 parameter beta function (Eq. 3; Yan and Hunt, 1999) was fit as well (same method) to account for a reduction in LER at the higher temperatures:

$$LER_T = R_{max} * \left(\frac{T_{max} - T}{T_{max} - T_{opt}} * \frac{T}{T_{opt}}\right)^{\frac{T_{opt}}{T_{max} - T_{opt}}}$$

Eq. 3

Where T is the temperature (ºC) variable, R_{max} is the maximum rate (maximum LER, mm/day) at the optimal temperature (T_{opt} , ºC) and T is the maximum temperature (ºC) at which the rate is 0. Because there was no self-starting function for the model the starting values used were: 30 mm/day for R_{max}, 25 ºC for T_{opt}, and 35 ºC for T_{max}.

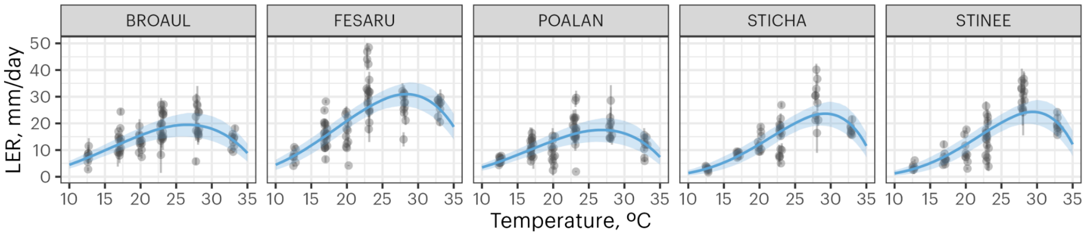

Figure S7. Fit of the beta model to the LER estimates (points with error bars for 95% confidence intervals) for the C3 species. Continuous line: model fit per species, bands: 95% confidence interval.

The beta model (Eq. 3, Figure S7) was a better fit than the logistic model for the C_3 species. Maximum LER was higher than that estimated by the logistic model's asymptote for all C_3 species. Still the differences between C_3 species were not just consistent with those shown by the logistic curves, but became even more pronounced as this model represented much better the shape of the response by including the decrease above the optimum temperature. The fitted curves were practically the same for *S. charruana* and *S. neesiana* (contrasting with the logistic model, Figures 1 and 2), and distinct from the other C_3 species (Figure S7): at the lower end of the temperature range (< 23 ºC), LER was lower in both *Stipa sp*. and the peak is more pronounced and at a slightly higher temperature (30 ºC rather than 27-28 ºC). The curves for *B. auleticus*, *F. arundinacea* and *P. lanigera* resulted in a similar shape, but *F. arundinacea* showed consistently higher LER values along the whole temperature range.

However, in crossing the predicted lines to estimate the crossover temperatures it did not have much of an effect, and the first (intrinsic response) classification is based on the bootstrapped linear response range from the logistic model (Figure S8). Therefore, for simplicity, this model was not included in the main text and crossover analysis.

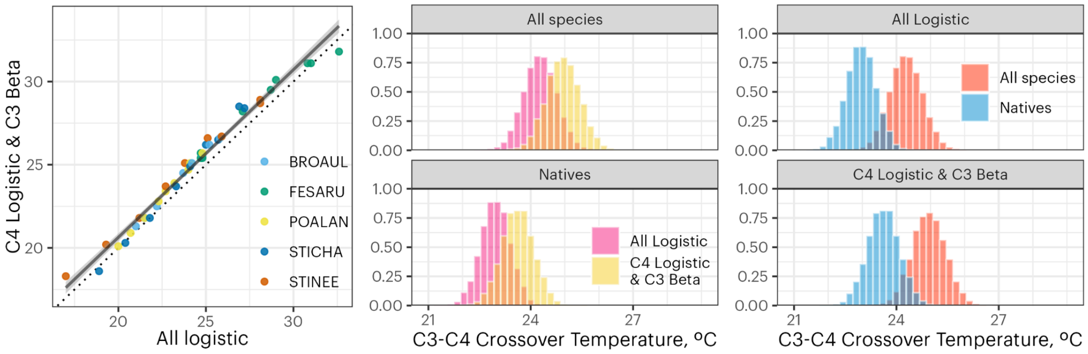

Figure S8. C_3-C_4 Crossover temperature analysis. First column: individual crossover temperature estimates using just the logistic model and both the logistic for the C_4 and beta for the C_3. Second and third columns: distribution of the bootstrapped crossover temperatures for both model combinations and for all species and natives only.

3. DISCUSIÓN

A pesar de que la temperatura afecta prácticamente a todos los procesos vegetales, las gramíneas perennes nativas (no domesticadas) suelen agruparse por su vía fotosintética para considerar la respuesta general a la temperatura. Sin embargo, hay razones para creer que no es el único factor que define la respuesta a la temperatura. Ajustamos curvas de respuesta a la temperatura en 14 gramíneas perennes de campo natural basándonos en la elongación foliar y encontramos que 1) hay diferentes respuestas incluso entre especies con la misma vía fotosintética y 2) que la temperatura de cruce C_3-C_4 es muy variable cuando se considera la tasa de elongación foliar (respuesta aparente). Además, modelar las respuestas como funciones continuas (rasgos dinámicos o de proceso) permite, no solo describir completamente el comportamiento de cada especie, sino también realizar una comparación precisa del comportamiento de las especies y mostrar cómo las diferencias también varían a lo largo del gradiente de temperatura.

3.1. RESPUESTAS INTRÍNSECAS Y APARENTES

En este trabajo diferenciamos dos niveles de respuesta a la temperatura: uno intrínseco, que refiere a qué temperaturas responden las especies (nicho térmico), y otro aparente, que incorpora la magnitud de la respuesta en los valores de TEF absolutos. La respuesta intrínseca permite caracterizar fisiológicamente a las especies, para comparar la actividad meristemática y las relaciones filogenéticas, mientras que la respuesta aparente es más adecuada para evaluar interacciones entre plantas —como la temperatura de cruce—, ya que incorpora otros efectos sobre el crecimiento y la TEF, como es el tamaño de las plantas y el largo final de las hojas. Al incorporar otra dimensión a la respuesta (magnitud) y más fuentes de variación que afectan también a la TEF, es coherente que encontráramos más divisiones al comparar las respuestas aparentes que las respuestas intrínsecas.

3.1.1. Temperatura de cruce

La temperatura media de cruce para la elongación foliar (~23-25 ºC) es consistente con la del modelo de rendimiento cuántico para las concentraciones atmosféricas de

CO_2 actuales de ~400-415 ppm (Ehleringer et al., 1997). Por lo tanto, al menos en promedio, las diferencias en la eficiencia fotosintética entre las vías C_3 y C_4 es un efecto importante (Atkinson et al., 2016). Sin embargo, al considerar los distintos pares de especies C_3 y C_4, otros factores que afectan al crecimiento foliar también adquieren relevancia, lo que se refleja claramente en la muy alta variabilidad en las temperaturas de cruce.

Por ejemplo, según el modelo de rendimiento cuántico fotosintético (Ehleringer et al., 1997), la temperatura de cruce para las monocotiledóneas C_4 NAD-me (como *C. dactylon*, Christin et al., 2009) debería ser mayor que la de los subtipos NADP-me (todas las demás C_4 nativas evaluadas; Christin et al., 2009). Pero, dado que consistentemente *P. notatum* se cruza casi a la misma temperatura que *C. dactylon* y que *A. affinis* también está siempre cerca (Figura 4B), el efecto de tener hojas más cortas parece más relevante que las diferencias entre subtipos de fotosíntesis C_4. Y, de hecho, es bien sabido que el tamaño del macollo tiene un fuerte efecto sobre la TEF y la LFF resultante (Arredondo y Schnyder, 2003; Berone et al., 2007; Casey et al., 1999; Kavanová et al., 2006). Entonces, para una determinada comunidad de pastizal, la temperatura de cruce C_3-C_4 dependerá de su composición botánica, lo que significa que la relación C_3-C_4 también tendría una estacionalidad particular para cada comunidad. Además podría ayudar a explicar, en parte, por qué en los campos uruguayos dominan las C_4 sobre las C_3, aún con temperaturas diurnas medias menores a la temperatura de cruce promedio y del rendimiento cuántico.

3.1.2. Exóticas

Según las curvas de TEF ajustadas, sería esperable que *F. arundinacea* domine estos pastizales, pero no es así. Por el contrario, aunque no se esperaría que *C. dactylon* prospere, es en realidad una de las invasoras más importantes en los Campos (Bresciano et al., 2014). ¿Cómo es posible esta discrepancia? Probablemente porque nuestro experimento no evaluó los efectos de los diversos factores ambientales que surgen en el campo, tanto abióticos como bióticos, y sus interacciones, que son relevantes para el éxito de las especies en el campo.

De hecho, en esta región el mayor riesgo para la persistencia de pasturas de *F. arundinacea* bajo pastoreo es la supervivencia estival de los macollos vegetativos formados en la primavera, probablemente por estrés térmico y no hídrico (Jáuregui et al., 2017; Jáuregui et al., 2024). Y, en parte, el éxito de *C. dactylon* como invasora resulta de ser una gran competidora por espacio y luz en comparación con las gramíneas perennes nativas, al favorecer el aumento de la biomasa aérea cuando se encuentra rodeado de otras plantas vecinas (García et al., 2023). Sin embargo, esta especie presenta un filocrón bastante corto (Lemaire y Agnusdei, 2000), lo que sugiere una estrategia que prioriza la producción de más hojas —aunque más cortas—, junto con el alargamiento de los entrenudos, lo cual explicaría, en parte, la discrepancia con nuestras curvas. No obstante, también es relevante que *C. dactylon* invade sistemas perturbados: antiguas chacras, campos degradados por el sobrepastoreo o intersembrados con leguminosas exóticas (por ejemplo, *Lotus sp.*) que compiten con las gramíneas perennes nativas estivales, especialmente en primavera (Jaurena et al., 2016); pero no logra invadir campos en buen estado y competir con plantas bien establecidas. Esto podría explicarse, en parte, porque en campos en buen estado los macollos son más grandes, por lo que logran TEF más altas y hojas más grandes que compiten mejor con una nueva planta por establecerse. A su vez, en campos degradados las gramíneas estivales nativas tendrían macollos más pequeños con menores TEF y hojas más chicas con menor capacidad de competir con el establecimiento de *C. dactylon*.

3.2. FILOGENIA Y LA HIPÓTESIS EVOLUTIVA

Una de las principales hipótesis para las diferentes respuestas a la temperatura entre plantas se basa en los orígenes evolutivos de cada especie y en una adaptación heredada a las condiciones del pasado (Chirstin et al., 2009; Edwards y Still, 2008; Parent y Tardieu, 2012; Taylor et al., 2010). De hecho, todas las especies C_3 del experimento pertenecen a la subfamilia Pooideae (la más numerosa y también reconocida por crecer a temperaturas más bajas que otras gramíneas C_3 dentro y fuera del clado BEP, Das et al., 2021; Schubert et al., 2019a; Sage et al., 2011; Vigeland et al., 2013; Zhong et al., 2018), mientras que las especies C_4 pertenecen a la subfamilia Panicoideae (las dos subfamilias más ricas en estos pastizales, Biganzoli y Zuloaga, 2015), excepto la introducida *C. dactylon* que pertenece a Chloridoideae (Gallaher et al., 2022; Soreng et al., 2017). Por lo tanto, las diferencias generales entre las especies C_3 y C_4 en este estudio (que las C_3 responden a temperaturas más bajas y alcanzan valores máximos de TEF más bajos) podrían atribuirse, en parte, a un efecto filogenético (Edwards y Smith, 2010; Edwards y Still, 2008).

Además, entre las C_3, las especies que responden a temperaturas más bajas (*B. auleticus*, *F. arundinacea* y *P. lanigera*) pertenecen al clado Core Pooideae, mientras que las que responden a temperaturas más altas (*S. charruana* y *S. neesiana*) pertenecen a la tribu Stipeae, que divergió c. 60 Mya (Schubert et al., 2019a; Soreng et al., 2017; Zhang et al., 2022). Los registros más antiguos de Pooideae en pastizales sudamericanos son de ~39 Mya (Strömberg et al., 2013), por lo que Stipeae y Core Pooideae ya llevaban bastante tiempo en caminos evolutivos independientes a su llegada a Sudamérica. El mismo patrón se observó también en la Pampa inundable, entre *S. neesiana* y otras especies coexistentes de Core Pooideae para la TEF total del macollo (Lemaire y Agnusdei, 2000). Y, de hecho, se ha demostrado que las gramíneas pertenecientes al Core Pooideae tienen una mayor carga y expresión de genes relacionados con la aclimatación al frío y la tolerancia a las heladas que las Stipeae (Schubert et al., 2019b). Aunque esto refiere a respuestas a temperaturas muy bajas y condiciones de estrés, demuestra la adaptación de estas especies a condiciones muy frías y, por nuestros resultados, parecería que también se traduce a poder crecer en temperaturas más bajas que otras especies.

Por el contrario, entre las especies $C_{4,}$ que son más recientes (los primeros registros en Sudamérica de gramíneas panicoides C_4 son de ~18 Mya, y no fue hasta alrededor de 9 Mya que las gramíneas C_4 se volverían dominantes en los biomas herbáceos, Edwards et al., 2010; Strömberg et al., 2013), no había patrones filogenéticos claros. Dentro de la tribu Andropogoneae, *C. selloana* responde a temperaturas más altas que *A. lateralis* y *E. angustifolius*. Y, lo que es aún más sorprendente, *P. notatum* responde a temperaturas más altas que el resto de las especies de Paspalum del experimento. Por lo tanto, las relaciones filogenéticas no siempre explican las diferencias observadas en las ventanas de crecimiento para estas especies, lo que contrasta con la hipótesis evolutiva (Edwards y Smith, 2010; Edwards y Still, 2008). Al mismo tiempo, el patrón observado entre las C_3 Pooideae (y la falta de dicho patrón en las C_4 Panicoideae) está en línea con la idea de que la evolución de respuestas divergentes a la temperatura lleva mucho tiempo teniendo en cuenta su complejidad, requiriendo la coordinación de muchos procesos (Parent y Tardieu, 2012, Parent et al., 2016). Pero entonces, ¿cómo puede ser que *C. selloana* y *P. notatum* presenten respuestas contrastadas con el resto de las gramíneas C_4 coexistentes? ¿Cómo es posible que estas dos gramíneas (relativamente no emparentadas) hayan evolucionado de esta manera en un período evolutivamente tan corto mientras que sus otros parientes más cercanos no lo hicieron?

Esto pone de manifiesto la relevancia de estudiar especies nativas (y no sometidas a procesos de selección y mejoramiento genético, Parent et al., 2016) y que coexisten y han evolucionado en un mismo ambiente (simpátricas) por lo que no están asociadas a gradientes ambientales, además frecuentes —incluso dominantes en algunos casos—, y de un sistema poco representado en la literatura internacional (el campo natural o los Campos).

3.3. FUTURO

Las diferencias que hemos observado se expresarán con mayor o menor intensidad en función de las condiciones de cada año o estación, por lo que futuros estudios deberían comprobar las interacciones con otras variables ambientales relevantes, como la disponibilidad de agua y nitrógeno; especialmente a temperaturas más elevadas. Además, ampliar la caracterización de la respuesta de la planta a la temperatura a un grupo mayor de especies podría ayudar a comprender el tiempo evolutivo necesario para la diferenciación de las respuestas de crecimiento de las hojas en comparación con la aparición del síndrome fotosintético C_4.

4.CONCLUSIONES

Al modelar la respuesta de la elongación foliar a la temperatura, encontramos que, efectivamente, las especies C_3 responden a temperaturas más bajas que las C_4; no obstante, no se trata en absoluto de grupos homogéneos de respuesta a la temperatura. Tales variaciones no se abordan adecuadamente en las clasificaciones funcionales existentes y no pueden atribuirse únicamente a las relaciones filogenéticas.

Las diferencias más contrastantes se dieron entre las especies C_3 (Pooideae), en las que las especies del Core Pooideae respondieron a temperaturas más bajas que las especies Stipeae. Sin embargo, entre las C_4 (Panicoideae) los contrastes no fueron tan fuertes, y las dos especies que responden claramente a temperaturas más altas son de tribus diferentes (Andropogoneae y Paspaleae). Además, dentro del género Paspalum, una especie se desvía notablemente del patrón de respuesta de las otras especies del mismo género.

Las respuestas a la temperatura son muy diferentes según cómo se evalúen. El tamaño de las plantas afecta la magnitud de la respuesta, y al considerarse puede cambiar la agrupación de las especies. Reconocer esto resulta muy relevante, sobre todo cuando se consideran las interacciones planta-planta; algo que queda bien claro en el amplio rango de estimaciones de temperatura de cruce. Curiosamente, las respuestas de temperatura LER de las dos exóticas no parecen explicar muy bien su presencia/éxito (o no) en estos pastizales, lo que demuestra también que, aunque la respuesta a la temperatura es extremadamente importante, hay otros mecanismos en juego a la hora de considerar el éxito y la distribución de las especies vegetales.

En general, estos resultados también demuestran el valor de los rasgos dinámicos o de proceso a la hora de describir la función de las especies en comparación a rasgos estáticos, sobre todo en los análisis comparativos entre distintas condiciones ambientales, como la temperatura.

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